Bulgarian Chemical Communications, Volume 50, Special Issue B, (pp. 123 – 129) 2018

Sulfide and nitrate driven fuel cell. Chemical and biochemical denitrification

S. Stefanov*, E. Razkazova-Velkova, M. Martinov, Ts. Parvanova-Mancheva, V. Beschkov

Institute of Chemical Engineering, Bulgarian Academy of Sciences, Acad. G. Bonchev, Str. Bldg.103, 1113 Sofia, Bulgaria

Received: July 14, 2017; Revised: November 8, 2017

A fuel cell is constructed for simultaneous sulfide oxidation and nitrate reduction. The results for biological and chemical denitrification in the cathode compartment are compared. The influence of different concentrations of sulfides and nitrates on the electrical power output of the fuel cell is examined, as well as their simultaneous neutralization. The electrodes used in the anode compartment are graphite rods and pyrolyzed paddling. The biological reduction of the nitrates is carried out by *Pseudomonas denitrificans* which increases the rate of nitrate depletion compared to the chemical fuel cell.

Keywords: Fuel cell, Sulfide oxidation, Denitrification, *Pseudomonas denitrificans*

INTRODUCTION

Wastewater is polluted with a variety of harmful substances, but we focused our efforts on hydrogen sulfide and nitrate pollution because of their high toxicity and detrimental environmental impact as a major prerequisite for acid rain. The sources of pollutants can be divided generally to natural and anthropogenic. Specifically for hydrogen sulfide the natural sources are volcanoes, thermal springs, closed deep water basins. The anthropogenic ones are associated with the petroleum, leather, pulp and textile industries, as well as sewage systems and wastewater treatment plants. The main processes for its neutralization are adsorption or absorption that can be combined with oxidation with strong oxidants [1-6], precipitation with metals [7] or biological oxidation [8]. Methods for thermal and electrical decomposition are also developed [9-11]. A classical method for hydrogen sulfide treatment is the Claus process but it requires high temperatures and specific and expensive catalysts [12]. Nitrate ion content in natural waters can be due to the excessive use of nitrogen composts or insufficient purification of wastewaters from households, industry and agriculture. After their reduction the nitrites produced are much more harmful for the animals' and humans' health. There are several established processes for treatment and neutralization of nitrate-containing waters, which can be generally divided as physicochemical and biological ones. Some of the main physicochemical methods are reverse osmosis, ion exchange, electrodialysis, distillation and activated carbon absorption [13-16]. Physicochemical methods are usually very expensive, especially when large quantities of wastewaters have to be treated and most result in high nitrate concentrated waters that can lead to additional problems concerning their follow up treatment. Biological denitrification is regarded as a very perspective and efficient method [16, 17]. The conclusion that can be derived from this is that most of the techniques for elimination of these pollutants are energy consuming and expensive as they need large capital investment and have high exploitation cost. The aim of the present study is to utilize the energy of oxidation of sulfides and reduction of nitrates in a fuel cell harvesting electrical power simultaneously with wastewater treatment.

MATERIALS AND METHODS

The principle scheme of the fuel cell and the scheme of the experimental installation are shown in Fig. 1.

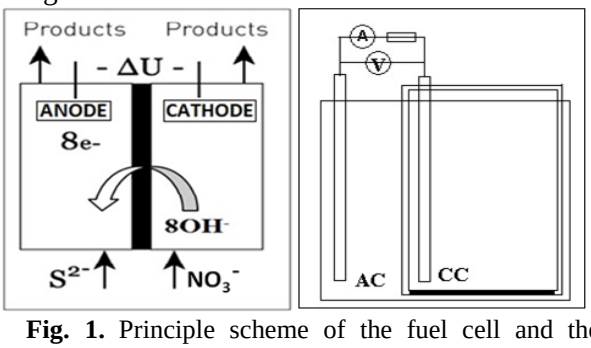

Fig. 1. Principle scheme of the fuel cell and the experimental installation

It consists of two concentrically situated compartments with effective volume of 300 ml each. The membrane (Celgard® 3501, S = 0.002 m^2, Table 1) is placed on the bottom of the inner one. The outer volume is the anode compartment (AC) and the inner one is the cathode one (CC). The electrodes used in the anode compartment are five standard cylindrical graphite rods (d=0.006 m,

* To whom all correspondence should be sent.
E-mail: mitovmario@swu.bg

Table 1. Characteristics of the membrane Celgard® 3501

Membrane	Type	Material	Thickness	Resist.(Ω.cm^2)	Purpose
CelGard® 3501	Anion	Polypropylene	25 μm	2.55	Alkaline battery separator

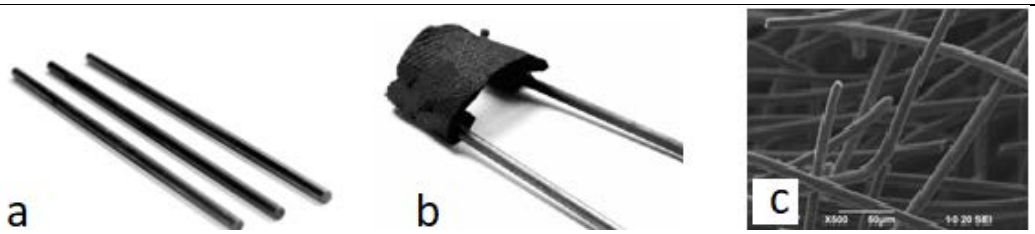

Fig. 2. Electrodes used in the fuel cell: a) Graphite rods, b) Paddling of activated carbon; c) SEM of a paddling of activated carbon.

L = 0.02 m, S=0.003 m^2) with total working surface of 0.015 m^2 (5 x 0.003 m^2) or pyrolyzed paddling of activated carbon with the same geometrical surface.

The pyrolyzation with simultaneous activation of the paddling is done by a patented technology [18] A photograph of the electrodes and SEM images of the paddling are presented in Fig. 2.

In some of the experiments 120 g (300 ml) of activated carbon (Fujikasau®, Japan, 680 m^2.g^{-1}.) were added in the cathode compartment in order to increase the electrode surface.

The feeding solutions were prepared by dissolving technical grade of $Na_2S \times 9\ H_2O$ and KNO_3.

The concentration of the sulfide solution was determined photometrically by converting the sulfide ion to methylene blue by addition of N,N-p-phenylenediamine [19], and the concentration of nitrates – by UV photometry by the method of Goldman & Jacobs [20].

The assumed reactions are as follows:

Anode:

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

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

Total:

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

Cathode:

$$2\ NO_3^- + 2\ H_2O + 4\ e^- \rightarrow 2\ NO_2^- + 4\ OH^-$$

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

Total:

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

The intermediates of both reactions (sulfites and nitrites) and the product of the anode reaction (sulfates) are monitored qualitatively. By adding $BaCl_2$ to the anode solution in the presence of sulfite and sulfate ions opalescence appears due to formation of precipitates of $BaSO_3$ and $BaSO_4$. By adding 2M HCl the $BaSO_3$ dissolves and any residual opalescence is due to the presence of sulfate (SO_4^{2-}) ions. In the presence of nitrites the addition of KI or $KMnO_4$ in an acid media gives ь colorful reaction for the former or decolorizes the latter.

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 [21]. In biological denitrification, the bacteria use nitrates as electron acceptor 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:

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow NO_2 \rightarrow N_2$$

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 [22, 23]

Studies were conducted with free and immobilized cells. The growth of the culture was monitored by using UV-spectrophotometry at λ = 660 nm. The growth curve is presented in Fig. 3. After a long period of lag-phase an exponential part of growth is observed that matches the denitrification phase.

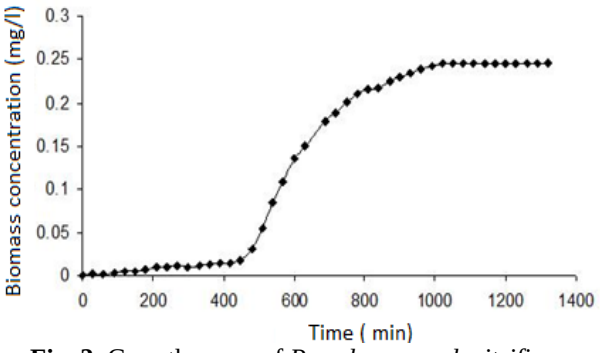

Fig. 3. Growth curve of *Pseudomonas denitrificans*

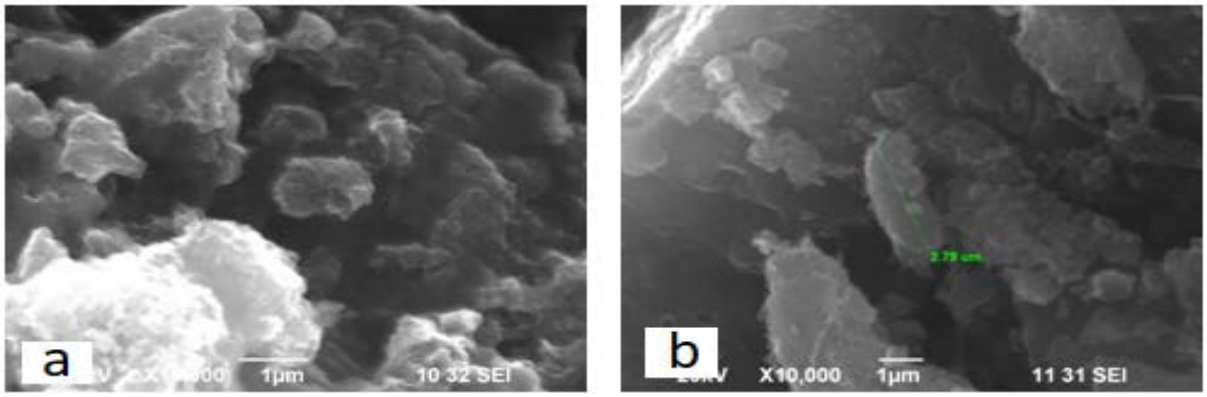

Fig. 4. SEM of a) Activated carbon (Fujikasui, Japan), b) Immobilized *P. denitrificans* on activated carbon (Fujikasui, Japan)

The activated granular carbon (Fujikasui®, Japan, 680 $m^2.g^{-1}$) was chosen as a support for immobilization due to the fact that microbial cells are easily attached to its surface. It has the added benefit to absorb toxic components, decreasing their concentration to tolerable values for microorganisms so that substrate and product inhibition is avoided, allowing the cell to operate at higher pollutant concentrations [24]. Additionally it has good electrical conductivity and very high specific surface area, making it an excellent electrode. The immobilized cells compose 2% of the mass of the activated carbon. SEM images of the activated carbon with and without immobilized cells are given in Fig. 4.

RESULTS AND DISCUSSION

Influence of type of electrodes in anode compartment

Previous studies by our team on a fuel cell for sulfide oxidation [25-28] show that optimal electrical and chemical results are obtained by using activated carbon as an electrode in the cathode compartment due to the large specific surface area the material provides. The effects of activated carbon as an electrode in the anode compartment are negligible compared to standard graphite rods hence an alternative was developed in the form of pyrolyzed paddling (Figs. 2a and 2b). This configuration of electrodes was tested for the newly designed fuel cell for simultaneous oxidation of sulfides and reduction of nitrates with the results shown in Fig. 5. As the figure shows, using pyrolyzed paddling yields about 40% higher power for the first 2 hours and about 30% for the first 6 hours. The explanation for this, as well as the initial low electrical results, is that the paddling is an adsorbent and initially the two processes compete, resulting in reduction of the local concentration around the electrode and respectively the power. After the initial adsorption both the incoming and the already adsorbed sulfide ions participate in the process increasing the power output of the cell.

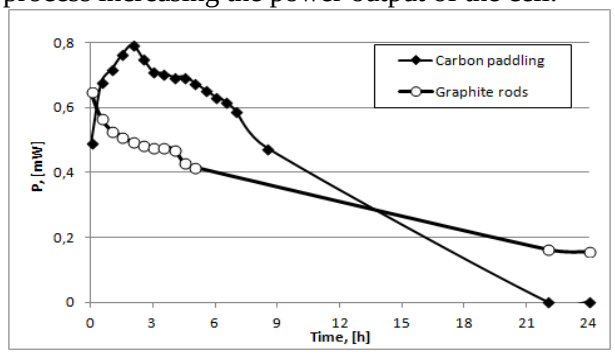

Fig. 5. Power in time for the tested electrodes

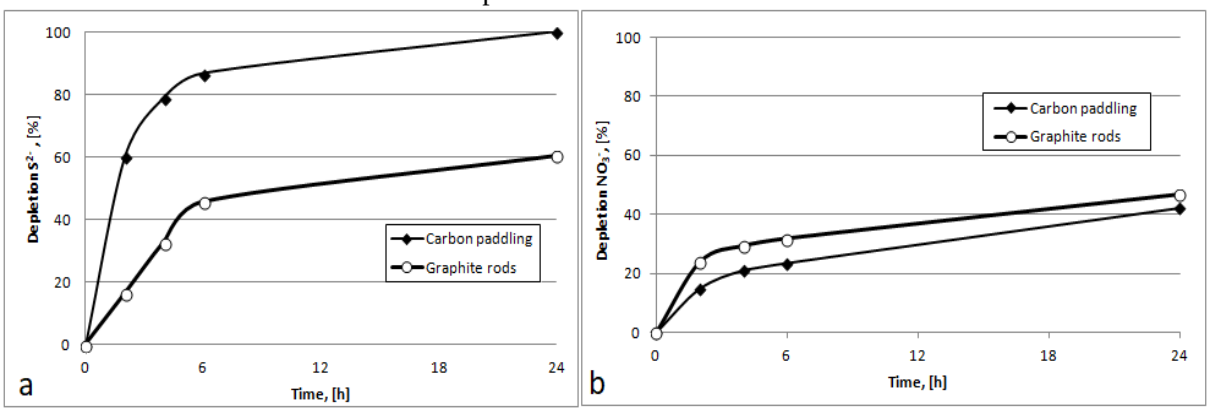

Fig. 6. Depletion in time of sulfides (a) and nitrates (b)

This is also confirmed by the sulfide depletion data shown in Fig. 6a. As can be seen, the paddling rapidly adsorbs about 40% of the sulfide and accordingly intensifies the oxidation process thus increasing the power and reaching total depletion of sulfide ions for 24 hours, which is not observed in the experiment with graphite rods. This higher process intensity also provides more electrons to the cathode compartment, which intensifies the nitrate reduction process as shown in Fig. 6b.

Influence of initial concentration

The effect of the initial concentration was studied as well. Experiments were conducted with two sets of totally different initial concentrations in the two compartments: C (S^{2-}) = 150 mg.l^{-1} and C (NO_3^-) = 200 mg.l^{-1} for the first experiment and C (S^{2-}) = 500 mg.l^{-1} C (NO_3^-) = 500 mg.l^{-1} for the second one. As can be seen from Fig. 7, the higher initial concentration provides higher power values.

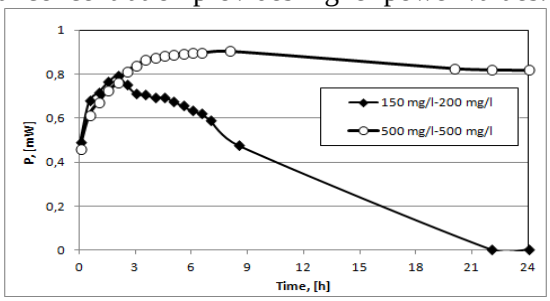

Fig. 7. Power in time for the tested initial concentrations

However, the capacity for oxidation of the fuel cell for sulfide ions (mg S^{2-}.h^{-1}) is limited. This is evident from the comparable power values for the first 2 hours. The "fuel" in the experiment with higher concentration is more, but the cell doesn't use it up fast enough to output more power. When the power of the lower concentration experiment decreases due to the depletion of the sulfide ions, the power in the other experiment is retained to values above the maximum for the other experiment. This is once again due to the constructive limitations for oxidation of large quantities of S^{2-} ions. This is also verified by the sulfide depletion data (Fig. 8a).

On the 24th hour mark the sulfide concentration in the second experiment is higher than the initial one for the first experiment. By calculating the amount of processed sulfides for 24 hours we can summarize that the fuel cell oxidizes 45 mg S^{2-} for 24 hours (from 45 mg total S^{2-}, 100% conversion) and 75 mg S^{2-} for 24 hours (from 150 mg total S^{2-}, 50% conversion). By applying similar calculations for the reduced nitrates shown in Fig. 8b we can summarize that the fuel cell reduces 25 mg NO_3^- for both experiments (from 60 mg total NO_3^-, ~40%, and 150 mg total NO_3^-, ~17%).

This allows us to assume that the maximum capacity for processing sulfides and nitrates for 24 hours of this fuel cell size using this size and type of electrodes is 75 mg of sulfides and 25 mg of nitrates.

Fuel cell with free culture and different electrodes

One of the most promising and cheap ways for intensifying the process is the use of microorganisms. So far we have had difficulties with the use of microorganisms that promote sulfide oxidation, but we have successfully used nitrate-reducing bacterial culture – *Pseudomonas denitrificans* (strain NBIMCC 1625).

Experiments are conducted with free microorganism culture in the cathode compartment. Graphite rods are used as electrodes in contrast to the chemical cell. This is due to the impossibility to use bulk activated carbon or pyrolyzed paddling as the free culture would be immobilized on them. As such limitations are not present for the anode compartment graphite rods and pyrolyzed paddling are used as electrode as in previous experiments. The results are shown in Fig. 9.

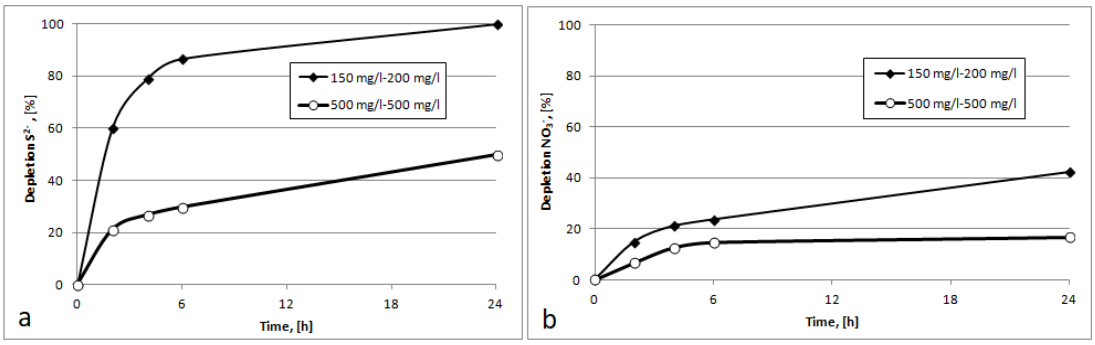

Fig. 8. Depletion in time of sulfides (a) end nitrates (b)

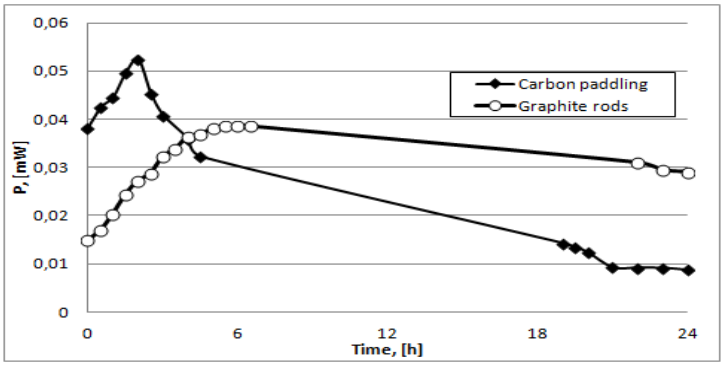

Fig. 9. Power in time for the tested electrodes

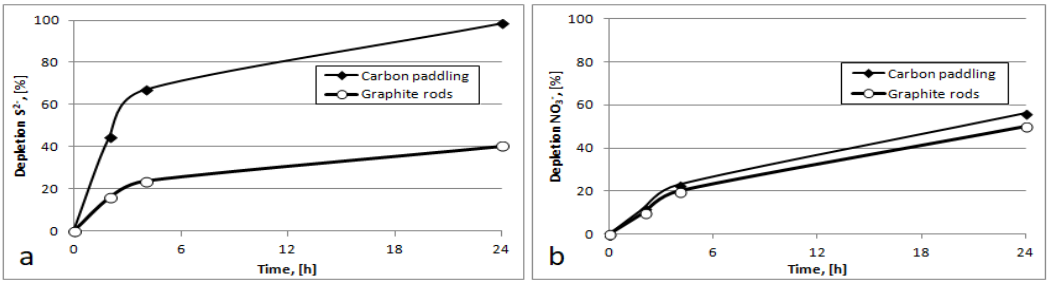

Fig. 10. Depletion in time of sulfides (a) end nitrates (b)

As can be seen, the course of the curves is similar to those obtained by the chemical cell, but due to the use of electrodes with much smaller effective surface area in the cathode compartment the power obtained is by one order of magnitude lower. The rate of depletion of sulfides (Fig. 10a) is faster when the more efficient pyrolyzed paddling electrodes are used. The depletion of nitrates is increased by 10-15% (Fig. 10b) compared with the chemical cell as a result of the action of bioculture. Even though the results are better our expectations were higher in regard to the nitrate oxidation and that is the reason we worked on improving the performance of the microorganisms by immobilizing them. According to the literature [21] the immobilized culture is more resistant to concentration fluctuations and reduces more nitrates per unit of
time compared to the free one.

Fuel cell with immobilized cells.

The most commonly used method of intensifying the work of biocultures is to immobilize them on a suitable support. One of the most conventionally used supports is activated carbon due to the combination of chemical and physical properties of the surface, improving the fixing on the bed and promoting the development of the culture as well as its low cost. For us it has the added benefit of high electrical conductivity so the activated carbon can be used as an electrode with which we have already achieved excellent results. Data on experiments with the immobilized bioculture on the activated carbon used for the electrode in the cathode compartment and the pyrolyzed paddling in the anode compartment for two totally different initial concentrations of sulfides and nitrates are given in Fig. 11. The fuel cell shows very good power output for both initial concentrations. The rate of depletion of S^{2-} (Fig. 12a) is also high, 100% for the lower initial concentration and 40% for the higher one. The reason for the poorer oxidizing performance in spite of the high power output is that the constructive limits of the capacity for oxidizing are reached in the second experiment.

The performance of bioculture in nitrate depletion (Fig. 12b) is excellent and within 90-95% for both concentrations. This is an inherent advantage of microorganisms – when there is more "food" they develop exponentially until the resource is depleted.

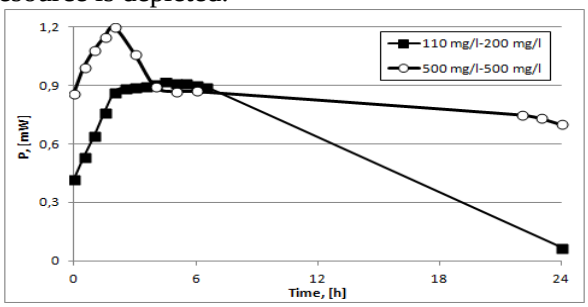

Fig. 11. Power in time for immobilized cells in the tested initial concentrations

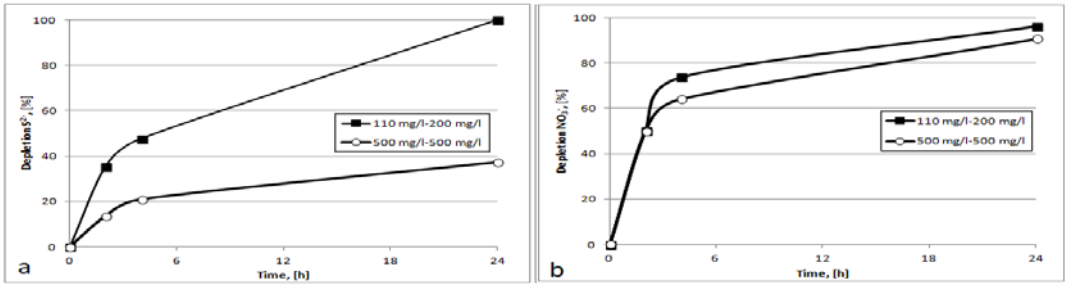

Fig. 12. Depletion in time of sulfides (a) end nitrates (b)

Comparison of biological and chemical denitrification

Experiments were carried out to compare the chemical and biological (with microorganisms in the cathode compartment) fuel cells of the same design, electrodes and high initial concentrations that are challenging for neutralizing them to harmless components. As shown in Fig. 13, the electrical power is high, with both cells delivering about 0.9 $mW.h^{1-}$(about 20 mW for 24 h). Regarding the purification of sulfides (Fig. 14a), the chemical cell performs better with 50% S^{2-} depletion for 24 hours, while for the biological cell the depletion is 37% (75 mg oxidized S^{2-} for the former compared to 55 mg S^{2-} for the latter (from 150 mg total)). Regarding the depletion of nitrates (Fig. 14b) the fuel cell with immobilized culture has drastic advantage with 90% to 17% for the chemical cell (135 mg reduced NO_3^- for the former compared to 25 mg NO_3^- for the latter (from 150 mg total)).

CONCLUSIONS

The co-treatment of wastewater contaminated with sulfides and nitrates with simultaneous generation of electric energy is feasible with a fuel cell of our own design.

The energy obtained in both types of fuel cells (chemical and biological) is stable and is within the range of 0.9 $mW.h^{-1}$ for the duration of the experiment.

With regard to the purification of sulfides, both cells operate similarly but have constructive limitations and can deal with contamination up to 75 mg / 24 h.

In terms of nitrate purification, the biological cell is reducing 5 times more pollutant than the chemical one – 135 mg / 24 h for the former compared to 25 mg / 24 h for the latter.

The comparison between chemical and biological fuel cell shows that one of the promising ways to intensify the purification process is the use of microorganisms.

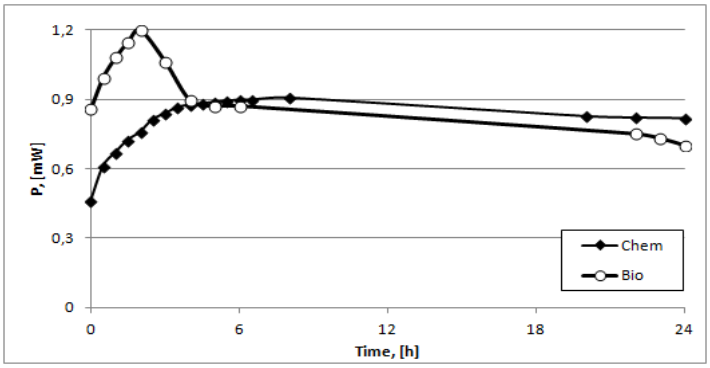

Fig. 13. Power in time for the tested different type fuel cell

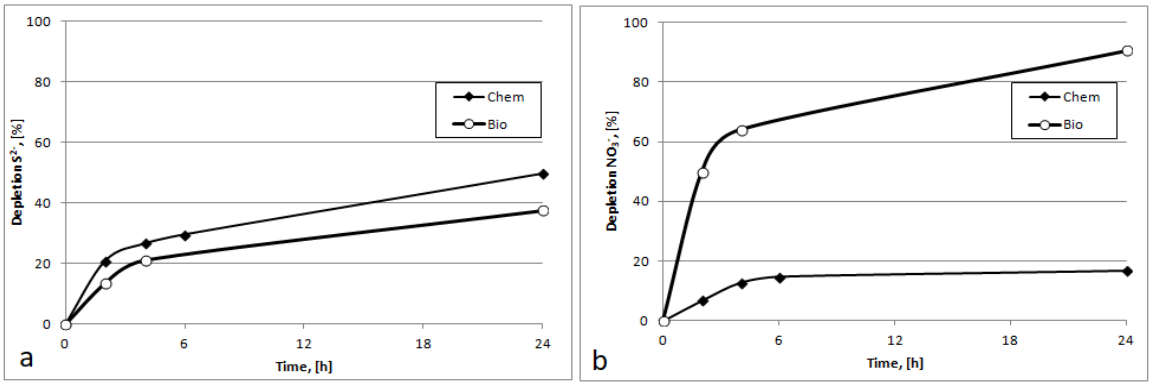

Fig. 14. Depletion in time of sulfides (a) end nitrates (b)

Acknowledgements: *This work was accomplished within the project E 02/15 – 12. 12. 2014 National Science Fund, Ministry of Education, and Science, Republic of Bulgaria.*

REFERENCES:

1. R. Cotrino, A.D. Levine, P. Amitzoglou, J. S. Perone, *Florida Water Resourses Journal*, pp. **22-25**, (2007).
2. M. Seredych, T.J. Bandosz, *Chem. Eng. J.*, 12859, (2007).
3. R.Wang, *Sep. Purif. Technol.* **31,** 111, (2003).
4. S. Yasyerli, I. Ar, G. Dogu, T. Dogu, *Chem. Eng. Process.*, **41 (9)**, 785 (2002).
5. A. T. Lemley, J. J. Schwartz, L. P. Wagenet, *Fact Sheet* **7**, Water treatments note, January 1999.
6. M. Tomar, T.H.A. Abdullah, *Water Res.*, **28**, 2545 (1994).
7. M. Henze, P. Harremoe, J. la Cour Jansen, E. Arvin, Wastewater Treatment – Biological and Chemical Processes, editors, second ed. Springer, Berlin, 1997.
8. Y. Kodama, Y. Watanabe, *Appl. Environ. Microbiol.*, **69**, 107 (2003).
9. G. Özgül, A.S. Koparal, Ü.B. Öğütveren, *Separation and Purification Technology*, **62**, 656 (2008).
10. J. Zaman, A. Chakma, *Fuel Processing Technology*, **41**, 159 (1995).
11. Y. Wang, Y. Heqing, W. Efeng, *Journal of Electroanalytical Chemistry*, **497**, 163 (2001).
12. M. Capone, in: J.K. Kroschwitz, M. HoweGrant (eds.), *Encyclopedia of Chemical Technology*, vol. **23**, Wiley, New York, p. 432, 1997.
13. A. Mautner, T. Kobkeatthawin, A. Bismarck, *Resource-Efficient Technologies*, **3,** 22 (2017).
14. A. Bhatnagar, E. Kumar, M. Sillanpää, *Chem. Eng. J.*, **163**, 317 (2010).
15. A. Bhatnagar, M. Sillanpää, *Chem. Eng. J.*, **168**, 493 (2011).
16. M. Shrimali, K.P. Singh, *Environmental Pollution*, **112**, 351 (2001).
17. S. Xia, F. Zhong, Y. Zhang, H. Li, X. Yang, *Journal of Environmental Sciences*, **22 (2),** 257 (2010).
18. L. Ljutzkanov., A. Atanasov, BG patent **№** 63594 /26.06.2002.
19. T.D. Rees, A.B. Gyllenpetz, A.C. Dochery, *Analyst*, **96**, 201 (1971).
20. E. Goldman, R. Jacobs, *J. Am. Water Works Assoc.*, **53**, 187 (1961).
21. Ts. Parvanova-Mancheva, Ph. D Thesis, 2009.
22. L. Foglar, F. Briski, L. Sipos, M. Vukovic, *Bioresource Technol.*, **96**, 879 (2005).
23. J.-H. Wang, B.C. Baltzis, G.A. Lewandowski, *Biotechnol. Bioeng.*, **47**, 26 (1995).
24. V. Beschkov, Biocatalysis Research Progress, editors Francesco H.R., Andrea R., *Published by Nova Science Publishers, Inc.*, Chapter XII, p. 281-305, (2008).
25. E. Razkazova-Velkova, M. Martinov, S. Stefanov, V. Beschkov, *Proceedings of the Georgian National Academy of Sciences, Chemical Series*, **42 (3)**, 258, 2016, ISSN:0132-6074.
26. M. Martinov, E. Razkazova-Velkova, S. Stefanov, *Journal of International Scientific Publications: Ecology & Safety*, **10**, 246 (2016), ISSN:1314-7234.
27. M. Martinov, E. Razkazova-Velkova, V. Beschkov, *Scientific Works of University of Food Technologies - Plovdiv*, LX, **1**, UFT Academic Publishing House, Plovdiv, p. 1046, 2013, ISSN:1314-7102.
28. E. Razkazova-Velkova, M. Martinov, L. Ljutzkanov, N. Dermendzhieva, V. Beschkov, *Scientific Works of University of Food Technologies - Plovdiv*, LX, **1**, UFT Academic Publishing House, Plovdiv, p. 1091, 2013, ISSN:1314-7102.