Bulgarian Chemical Communications, Volume 50, Special Issue B, (pp. 147 – 153) 2018

Identification of bacterial community in a sediment microbial fuel cell

S. Hristoskova[1], I. Bardarov[2], D. Yankov[1*], S. Danova[3], Y. Hubenova[4], M. Mitov[1]

[1] *Institute of Chemical Engineering – BAS, Sofia, Bulgaria*
[2] *Department of Chemistry, South-West University "Neofit Rilsky", Blagoevgrad, Bulgaria*
[3] *The Stefan Angeloff Institute of Microbiology – BAS, Sofia, Bulgaria*
[4] *Institute of Electrochemistry and Energy Systems "Acad. Evgeni Budevski" – BAS, Sofia, Bulgaria*

Received July 30, 2017, Revised November 3, 2017

The aim of the present study was to investigate the bacterial community structure in a single-chamber sediment fuel cell (SMFC). The SMFC was prepared with sediment and water from Struma River and was functioned more than three years before analysis. Samples were taken from different places from cathode and anode compartments as well as from both electrodes. A protocol for microbes' isolation and characterisation has been adapted, using classical microbiological methods and different selective media. The genomic DNA was isolated from pure microbial cultures, obtained from selected single colonies and cultivated twice for biomass accumulation. The obtained DNA samples were analyzed by 1% v/v agarose gel electrophoresis and the concentration and purity were checked. The DNA was used as a target in the PCR assay and following 16 rDNA sequencing analysis. The obtained sequences were analyzed with BLAST analyses, and species identification of the strains were performed .

Keywords: sediment microbial fuel cell, bacterial community, PCR analysis, identification.

INTRODUCTION

In the present days our society facing the threat of exhausting fossil fuels. According to predictions of British Petroleum [1] global proved oil reserves would be sufficient to meet 50.6 years of global production at 2016 levels. In view to decrease the dependence of contemporary economic from petrol and other fossil fuels, the researchers are focused on alternative energy sources. Still solar, wind, biomass-based and other systems for alternative energy generation are not enough economically effective because of their low effectiveness [2]. Nevertheless, the hopes for overcoming greenhouse gas and CO_2 emissions problems are put on alternative energy. A promising source of alternative energy represents fuel cells and there is abundant literature on it [2-6].

Microbial fuel cells (MFC) are kind of fuel cells in which biological redox ability of the microorganisms are combined with electrochemical reactions with the purpose of electricity production. An advantage of MFC is the possibility to combine the electricity production with waste water treatment or utilization of various waste biomass as substrate. The problems and progress in MFC utilization are discussed elsewhere [7-10].

Sediment microbial fuel cells (SMFC), also known as benthic MFC, are a kind of MFC, attracting much attention recently because of their unique properties. First of all is a very simple construction and exploitation due to lack of membrane. Another specific trait is that SMFCs work exclusive under anoxic conditions. Production of electricity in SMFC is usually coupled with environmental protecting processes as soil bioremediation from heavy metals [11, 12], hydrocarbons [13, 14] or other organic pollutants [15, 16].

The conversion of the chemical energy stored in an organic substrate to electro-energy can be realized by the aid of electrochemically active microorganisms (EAM). Assimilating the substrate (electron donor) the EAM produce electrons, which are transferred to the anode and by an external electrical circuit are leaded to the cathode where the process of oxidizing of an electron acceptor take part.

There are two main mechanisms for electron transport – direct electron transfer (DET) and indirect (mediated) electron transfer (MET) [9]. For realization of DET it is necessary that EAM have direct contact to the anode surface – either by the cytochrome *c* proteins of the outer cell's membrane or by bacteria's pili. Having in mind that EAM are prone to form a bacterial film on the anode it has to be the preferential choice. MET is characterized with presence in the system of a redox mediator, synthesized by the microorganisms or artificially added. Especially for SMFC, it is impossible to distinguish the type of electron transport mechanism due to the great variety of microorganisms present in the biofilm.

For the effective operation of an SMFC it is of great importance microbial diversity in the electrode's biofilms to be known, so the researcher

* To whom all correspondence should be sent.
E-mail: yanpe@bas.bg

to concentrate his efforts towards the EAM process conditions optimization. Knowledge of the type of microorganisms present in the biofilm will give the possibility to construct an MFC with a pure culture of a single microorganism or to work with a consortium of well-defined EAM.

In a review paper on the fundamentals of benthic MFC Girgius *et al*. [17] summarized among others the data for microbial ecology in sediment MFC. The authors pointed out that in general, two major phylogenetic groups from the anode biofilm have implicated the power production: the δ- and γ-*Proteobacteria*. δ-*Proteobacteria* are ubiquitous in marine sediments, and are involved in sulfate reduction via the oxidation of organic matter or hydrogen. On the genus level the identified microorganisms were predominantly allied to the genus *Desulfuromonas*. The studies of biofilm formation on cathode revealed high representation of γ-*Proteobacteria* allied to *Pseudomonas flourescens*, capable of electron transfer via quinones.

García-Muñoz *et al*. [18] constructed an SMFC with water and sediment from Rio Tinto River. Studying the microorganisms presented in this acidic ecosystem the authors identified and quantified the acidophilic microorganisms that had colonized the anode and cathode surfaces. The dominant species on the anode surface belonged to the *Acidiphilium* genus. Minor quantities of *A. ferrooxidans* and *Leptospirillum* spp. were detected on the anode. These aerobic iron oxidizers were detected mainly on the cathode surface.

Abbas *at al*. [12] observed that after 120 days of operation the biofilms on graphite cathode and anode of an aerated and a nonaerated cathode SMFC were dominated mostly by *Pseudomonas* spp. The SEM observation revealed the difference in size of anode and cathode biofilms. The aerated SMFC gave higher power generation and toxic metal removal than nonaerated one. Majdumner *et al*. [19] studied a sediment microbial fuel cell with an air-cathode system with cotton clot electrodes. The biofilm of the anode of the SMFC was governed by γ-*Proteobacteria*.

Piscotta *et al.* [20] used SMFC for enriching graphite fiber brush anodes using two different marine sediments (from Chesapeake Bay, Annapolis and from steel piling in the Baltimore Inner Harbor). After establishment of electroactive biofilms on the anodes, they were electrically inverted to function as cathodes in two-chamber bioelectrochemical systems. Bacterial colonies were isolated from electron-accepting brushes and were analyzed. There were substantial differences in the microbial consortia on the two biocathodes with the highest current densities. The Harbor biocathode film primarily consisted of bacteria most similar to *Eubacterium limosum*, *Desulfovibrio* sp., and *Gemmata obscuriglobus,* while the Chesapeake Bay cathode film is dominated by *G. obscuriglobus (*also identified on the Harbor cathode), and members of three different genera, *Mesorhizobium*, *Rhodococcus*, and *Azospirillum*.

Sacco *et al*. [21] have studied the performance of SMFCs constructed with sediment from Río de La Plata River. Graphite disks and rods were used as electrodes. Three different types of SMFCs were made: non-current control SMFCs, SMFCs amended with sodium acetate and SMFCs made without exogenous addition. Bacterial community analysis showed that anodic biofilms of SMFCs fed with sodium acetate were dominated by *Shewanella* sp., *Pantoea* sp. and *Pseudoalteromonas* sp., while these of SMFCs without exogenous addition were dominated by different species of the *Bacillus* genera.

In the work of Erable et al. [22] the authors studied an SMFC with marine sediment and a carbon (bioanode) and a stainless still (biocathode) electrodes. The microbial diversity of biofilms formed reveled that *α-Proteobacteria* and *δ-Proteobacter*ia were predominant in the biofilm collected from the biocathode, while *α-Proteobacteria* and *Bacteroidetes* were mainly present inside the biofilm from the bioanode. On the genus level *Sulfitobacter* sp. (*α-Proteobacteria*) were found in both anodic and cathodic biofilms.The representatives of the same genus are presented also in the sediment but there are representatives of genus *Clostridium, Bacillus* and *Sporosarcina*.

From the short literature review above, it is evident that the microbial community structure depends on the origin of sediment used, type of the electrodes, electron donors and operation time.

The aim of the present study was to identify the microbial community in a river sediment MFC after stable work for more than three years.

MATERIALS AND METHODS

Construction of single-chamber sediment fuel cell.

The fuel cell was constructed using cylindrical plastic vessel. Nearly a half of the vessel was filled with sediment (collected from the basin of Struma River near Blagoevgrad (GPS coordinates: 42.051209, 23.076744). The anode (Graphite disk, 6 cm diameter, 1 cm thickness; GES Co., apparent

density 1.68 g/cm³, porosity 24%, electrical resistance 6.0 μΩ.m) was placed into the sediment (at approximately 3 cm from the bottom). The sediment was covered with water (around one third of its volume) taken from the same place. The cathode, identical as the anode, was placed nearly to the sediment/water boundary. Samples were collected from a sediment fuel cell, working for more than three years without any additions of nutrients, except water for recovering the losses caused by evaporation.

Samples' collection

Samples were taken from the anode and cathode space as well as from both electrodes. From the anode space 3 samples of different heights were taken - from the surface, at a depth of 5 cm (near the anode) and from the bottom (see Fig.1). Each soil sample (about 50 g) was placed on a sterile cannula and homogenized in a sterile box, then an average sample of 5 g was removed by quartering. Each sample was divided into five 1 g portions. Samples of the sediment element were poured with 9 ml of sterile saline and homogenized well to extract the maximum amount of microorganisms. After sedimentation of the soil particles, the supernatant was transferred in sterile test tubes. From the cathode space two samples were taken - near the surface and the phase boundary. Samples were taken with sterile pipettes and placed in sterile vials for further processing. Separate samples were taken from the surface of the anode and the cathode. The electrodes are carefully rinsed with sterile deionized water and the biofilm was carefully transferred in a sterile bottles using a sterile scalpel. A total of 12 samples were taken from different parts of the two surfaces of the electrodes, each with an area of about 3 cm^2. Biofilm samples from both electrodes were placed in sterile saline and homogenized.

Microorganisms, Media and Culture conditions

1 ml of the collected samples were seeded in 9 ml of the below-mentioned media to grow the various microorganisms.

Heterotrophic saprophytic bacteria – in Meat Pepton Buiilon (MPB, BulBio) at 22 ºC for 72 h and at 37 ºC for 48 h;

Spore-forming bacteria – in MPB at 37 ºC for 48 h after a pre-luminary step of sample's heating at 80 ºC for 10 min;

Mycromycetes – in Czapek's medium 22 ºC for 72 h;

Actinomycetes – in Starch-ammonia medium at 22 ºC for 72 h;

Thermophilic bacteria – in MPB at 60 ºC for 48 h.

Serial dilutions were made from each collected culture, after cultivation at different temperature, then were plated on Petri dishes containing selective agar medium, under the same culture conditions. The single colonies, with different morphotypes were selected, described and re-inoculated into a liquid medium and cultivated at the same conditions. All isolates were stored at -20ºC in broth medium supplemented with 20% (v/v) glycerol.

For DNA isolation the biomasses of 10 ml exponential pure microbial cultures were harvested by centrifugation (5000 rpm, 5 min, Hermle centrifuge) and was frozen at -20 °C.

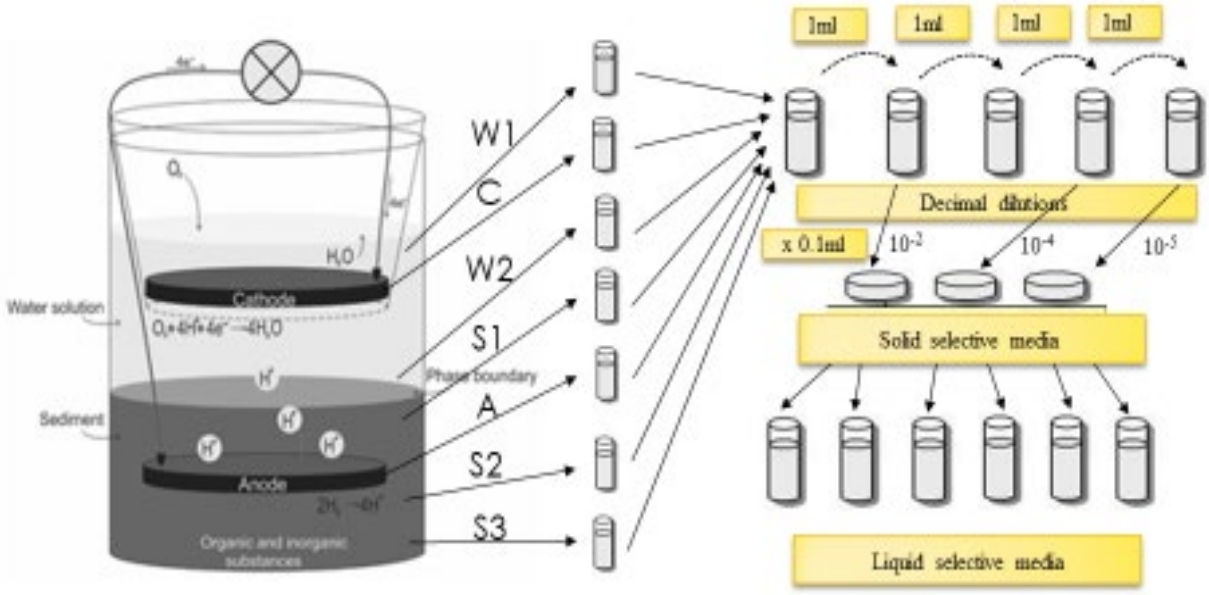

Fig. 1. Scheme of sediment fuel cell and samples' preparation.

Microbial community analysis

Total DNA from investigated isolates was extracted with ISOLATE II Genomic DNA Kit (Bioline) according the manufacturer's instructions. The amount and purity of the extracted DNA were determined by measuring the absorbance at different wavelengths by UV-Vis spectrophotometry.

The DNA concentration was determined at λ = 260 nm using the formula

$$1.0\ A_{260}\ \text{unit} = 50\ \mu g / \text{ml DNA}.$$

The purity of the extracted DNA was determined by measuring the absorbance at 230, 260 and 280 nm. Pure, unpolluted protein-labeled DNA has an A_{260} / A_{280} ratio equal to, or greater than 1.8 and an A_{260} / A_{230} ratio of at least 2.0.

The species identity of newly isolated microorganisms was determined by PCR amplification of the 16S rRNA gene and sequencing. All PCR reactions were done in a Progene cycler (Techne, UK) in 25 µl volume, using Ready To Go™ PCR beads (Amersham Biosciences).

Universal primers pair: forward fD1 (5`-AGAGTTTGATCCTGGCTCAG-3`) and reverse rD1 (5`-TAAGGAGGTGATCCAGGC - 3`) were used.

The PCR conditions were: initial denaturation for 5 min at 94 ºC followed by 35 cycles of denaturation at 94 ºC for 1 min, annealing at 56.5 ºC for 1,15 min and elongation at 72 ºC for 1,15 min; and a final elongation at 72 ºC for 5 min.

Obtained PCR products were visualized on 1% (w/v) agarose gel (Sigma). A DNA ladder molecular marker 100 bp is used as a standard for the visualization of the amplified fragments. The PCR products were purified and by Macrogen Inc., (Amsterdam) and compared with the nucleotide sequence in the Gene Bank database (http://www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Following the above described procedure totally 55 colonies were selected and further investigated. They possess different morphological characteristics and were isolated from selective/elective for different group of bacteria or *Micromycetes* agar plate media (Fig. 2).

A microscopic control was applied to verify the purity of all selected microbes. The domination of bacterial isolates v/s fungal ones into the group of selected 55 colonies have to be pointed.

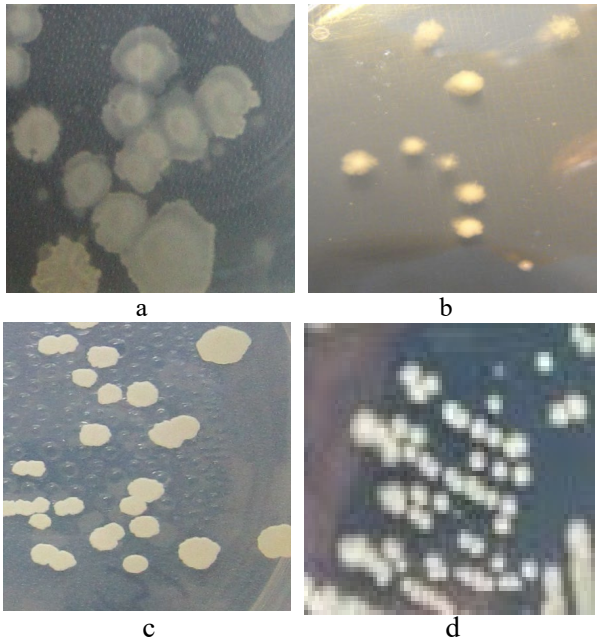

Fig. 2. Morphological characteristics of single colonies obtained on agar plates media, after cultiation of serial dilutions of the samples from SMFC.
The isolates were ccharacterised as
a) *Lysinibacillis* sp., b) *Clostridium* sp.,
c) *Paenibacillus* sp., d) *Pseudomonas* sp.

The species identification of bacterial strains was obtained by combining classical phenotypic and molecular methods. Isolated total DNA (Fig. 3) was used as template for PCR amplification of the 16S rRNA gene (Fig. 4), using universal primers.

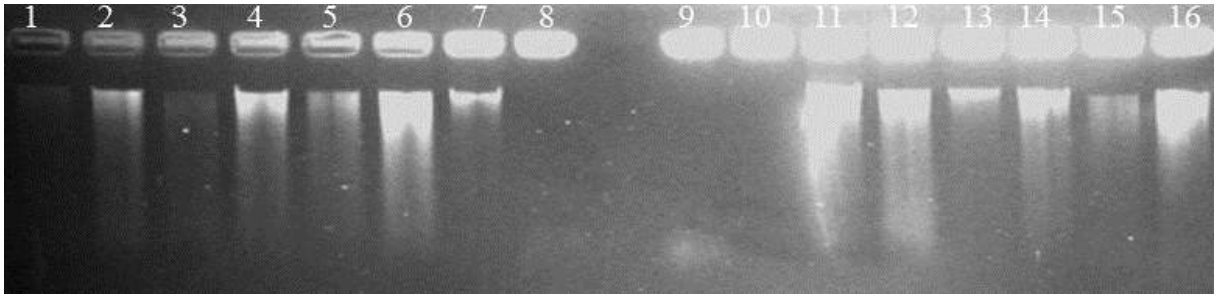

Fig.3. Visualization of some preparations of total DNA isolates. Gel electrophoresis (1% w/v Agarose Sigma); 10 min at 100 mV and 15 min at 90 mV; 20 min in ethidium bromide and 30 min in distilled water.
Starts: 1: MAA ; 2: MAA1; *3*: MAA2; 4: MA12; 5: MA13; 6: MA22; 7: MK11; 8: MK12; 9: MK13; 10: MK21; 11: MK22; 12: MK41; 13: MC14; 14: MC13; 15: MC21; 16: MC31.

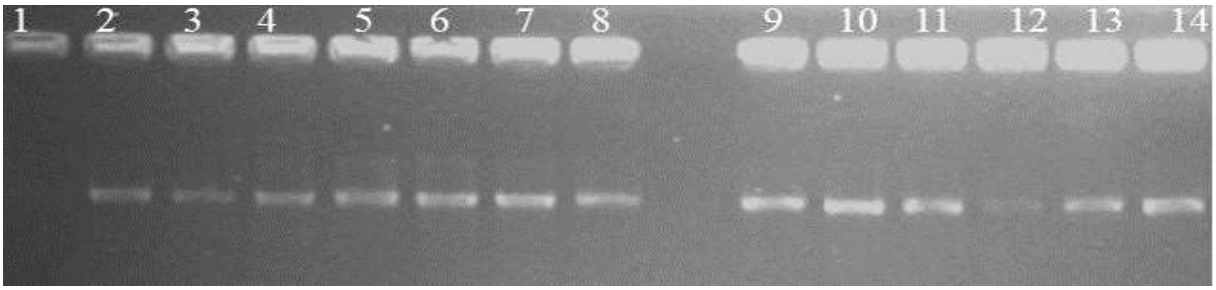

Fig.4. Visualization of the PCR products, obtained with universal primers for 16S rDNA analysis of isolates, in agarose gel electrophoresis (1 % w/v Agarose Sigma) after Ethidium bromide staining.

Starts: 1: MC31; 2: MC21; 3: MC14; 4: MC13; 5: MA22; 6: MA13; 7: MAA2; 8: MAA1; 9: MAA; 10: MA12; 11: MK11; 12: MK22; 13: MK14; 14: MC32 .

Obtained PCR products (~1550 bp) were visualized in 1 % (w/v) agarose gel (Sigma) and were sequenced in Macrogen, Inc Amsterdam.

The resulting sequences were further processed with the program *Chromas 2.3* (www.technelysium.com.au/chromas.html) and compared with the available nucleotide database from GenBank (http://www.ncbi.nlm.nih.gov) using the BLAST program).

From the selected 55 isolatees, 21 were identified – 3 on cathode, 7 on anode and 11 from sediment samples (Table 1). The majority of them (18) belongs to the phylum *Firmicutes* and three to the phylum γ-*Proteobacteria* (see Table 2). The representatives of the phylum γ-*Proteobacteria* were identified on the cathode

- *Pseudomonas oryzihabitans* strain h-2 and in the upper part of the sediment - *Pseudomonas koreensis* strain MLS-6-4 and *Pseudomonas putida* strain LCR80.

The other microorganisms on the cathode belong to the genus *Lysinibacillus*. The identified on the anode microorganisms are representative of the genus *Paenibacillus* and *Lysinibacillus*.

Table 1. Number of the isolated colonies and identified microorganisms.

Position	Isolated colonies	Identified microorganisms
W1	None	-
W2	8 colonies	0
K	9 colonies	3
A	12 colonies	7
S1	11 colonies	3
S2	7 colonies	3
S3	8 colonies	5

Table 2. List of identified microorganisms.

№	Sample	Species affiliation	Similarity	Position and medium
1	MK11	*Pseudomonas oryzihabitans*	85%	Cathode – MPA*; 37 °C
2	MK22	*Lysinibacillus fusiformis*	85%	Cathode - MPA; 22 °C
3	MK41	*Lysinibacillus boronitolerans*	86%	Cathode - MPA; 37 °C
4	MAA	*Lysinibacillus sphaericus*	87%	Anode – MPA; 37 °C
5	MAA1	*Lysinibacillus boronitolerans*	83%	Anode – MPA; 37 ^{0}C
6	MAA2	*Lysinibacillus fusiformis*	88%	Anode – MPA; 37 °C
7	MA12	*Paenibacillus dendritiformis*	85%	Anode – MPA; 37 °C
8	MA13	*Paenibacillus apiarius*	90%	Anode – MPA; 37 °C
9	MA22	*Paenibacillus apiarius*	89%	Anode – MPA; 37 °C
10	MA11	*Paenibacillus apiarius*	99%	Anode – MPA; 37 °C
11	CC1	*Pseudomonas koreensis*	100%	S1 – Chapek's medium; 22 °C
12	CC3	*Pseudomonas putida*	96%	S1 – Chapek's medium; 22 °C
13	MC12	*Bacillus cereus* .	98%	S1 – MPA; 37^{0}C
14	HC2	*Paenibacillus odorifer*	98%	S2 – SAA**; 22 °C
15	HC3	*Paenibacillus caespitis*	99%	S2 –SAA; 22 °C
16	MC23	*Bacillus cereus*	100%	S2 – MPA; 22^{0}C
17	MC3A	*Clostridium mangenotii*	98%	S3 – MPA; 22^{0}C
18	MC35	*Lysinibacillus macroides*	97%	S3 – MPA; 22^{0}C
19	MC36	*Lysinibacillus macroides* .	98%	S3 – MPA; 22^{0}C
20	MC3	*Lysinibacillus sphaericus* .	94%	S3 – MPA; 22^{0}C
21	MC31	*Sporosarcina luteola* ..	87%	S3 –MPA; 37 °C

*MPA-Meat Peptone agar, **SAA – Starch - ammonia agar.

Our results are in accordance with some recently publiched data.

In the paper of Jung *et al*. [23] the results of anode bacterial communities' studies of SMFCs with different anodes were presented. Four different anodes were used - a magnesium electrode, a magnesium electrode supplied with chitin particles, a graphite electrode, and a graphite electrode supplied with chitin particles. The reported results showed distinct difference in the anodic biofilms.

In a phylum level the magnesium anodes were dominated by *Proteobacteria* and *Firmicutes* in nearly equal proportions, while the graphite anodes were dominated solely by *Proteobacteria*. In a class level, anode bacterial communities were very different among the four anodes. Bacterial community of magnesium anode was most diverse and was comprised of *δ-Proteobacteria*, *Bacilli*, *Clostridia* and *γ-Proteobacteria*, whereas an anode bacterial community of magnesium/chitin anode was dominated by *Bacilli* and *α-Proteobacteria*. Anode bacterial communities of both graphite anodes were dominated by *δ-Proteobacteria*. The differences in bacterial communities between four anodes were most distinctive in species-level. In general bacterial communities of the chitin-absent anodes had larger richness and diversity than those of the chitin-supplemented anodes.

D. Khater *et al*. [24] have studied the performance of an air–cathode single-chamber mediator-less microbial fuel cell with electrodes made from carbon paper. MFC was seeded with mixed culture of aerobic activated sludge obtained from a municipal wastewater treatment plant. The analysis of microbial diversity on the electrode showed that the dominant phyla in the anodic biofilm are *Firmicutes, γ-Proteobacteria, α-Proteobacteria and Actinobacteria*.

Q. Zhao et al. [25] investigated SMFC with multiple anodes. The sediment was from lake origin. The microbial community structure in the sediment and anode biofilms were analyzed and it was found that the microbiota is dominated by the representatives of the phyla *Firmicutes, Proteobacteria* and *Euryarchaeota.*

Ueno and Katajima [26] analyzed an SMFC with a sediment created by mixing freshwater sediment from a fish breeding facility with andosol, zeolite and sand. While the initial sediment is dominated on the genus level by the representatives of *Thiobacillus*, microbial community analysis on the surface of the buried electrodes showed that the genus *Geobacter* had drastically propagated in a sample from the reactor where the electrodes were buried. Archaeal population had decreased to approximately 1/6 of its original level.

This study confirms the findings of other authors for the bacterial community in SMFC. The enrichment of the community with representatives of limited amount of species has also been previously reported.

CONCLUSIONS

The microbial community of investigated SMFC was dominated by the phylum *Firmicutes* with some representatives of the phylum γ-*Proteobacteria.* The most propagated are *Lysinibacillus* strains found on the cathode, the anode and in the sediment. *Paenibacillus* strains were isolated from sediment and from anode.

Anode bacterial communities of the graphite anodes were dominated by a few species - three strains of *Lysinibacillus* and two of *Paenibacillus*.

Future studies are planned to explore the possibility of using of isolated pure cultures as sole culture in SMFC.

Acknowledgements: *The authors are thankful to the National Science Fund of Bulgaria for the financial support by the contract DFNI E02-14/2014, which made this study possible.*

REFERENCES

1. BP Statistical Review of World Energy June 2017
2. V. Das, S. Padmanabanb, K. Venkitusamya, R. Selvamuthukumaran, F. Blaabjerg, *Renewable and Sustainable Energy Reviews* **73**, 1 (2017).
3. B.C. Ong, S.K. Kamarudina, S. Basri, *International journal of hydrogen energy*, **42,** 10142 (2017).
4. T. Sadhasivam, K. Dhanabalan, S.-H. Roh, T.-H. Kim, K.-W. Park, S. Jung, M. D. Kurkuri, H.-Y. Jung, *International Journal of Hydrogen Energy*, **42**(7) 4415 (2017).
5. R.K.A. Rasheed, Q. Liao, Zh. Caizhi, S.H. Chan, *International journal of hydrogen energy*, **42**, 3142 (2017).
6. Sh. Hossain, A.M. Abdalla, S.N.B. Jamain, J.H. Zaini, A.K. Azad, *Renewable and Sustainable Energy Reviews*, **79,** 750 (2017).
7. V.B. Oliveira, M. Simxes, L.F. Melo, A.M.F.R. Pinto, *Biochemical Engineering Journal*, **3**, 53 (2013).
8. M. Rahimnejad, A. Adhami, S. Darvari, A. Zirepour, S.-E. Oh, *Alexandria Engineering Journal*, **54**, 745 (2015).
9. C. Santoro, C. Arbizzani, B. Erable, I. Ieropoulos, *Journal of Power Sources*, **356**, 225 (2017).
10. V.G. Gude, *Journal of Cleaner Production*, **122**, 287 (2016)
11. S.Z. Abbas, M. Rafatullah, N. Ismail, M.I. Syakir, *Int. J. Energy Res*., **41**, 1242 (2017).
12. S. Z. Abbas, M. Rafatullah, N. Ismail, R.A. Nastro, *Int J Energy Res*, **41**, 2345 (2017).

13. M. Sherafatmand, H.Y. Ng, *Bioresource Technology*, **195**, 122, (2015).
14. X. Xu, Q. Zhao, M. Wu, J. Ding, W. Zhang, *Bioresource Technology*, **225**, 402, (2017).
15. G. Mohanakrishna, I.M. Abu-Reesh, S. Kondaveeti, R.I. Al-Raoush, Zh. He, *Bioresource Technology*, **253**, 16 (2018).
16. X. Xu, Q. Zhao, M. Wu, J Ding, W. Zhang, *Bioresource Technology*, **225**, 402 (2017).
17. P.R. Girguis, M.E. Nielsen, C.E. Reimers. "Fundamentals of Benthic Microbial Fuel Cells: Theory, Development, and Applications." In: Bioelectrochemical systems. First edition. Springer Verlag Press, 2010.
18. J. García-Muñoz, R. Amils, V.M. Fernández, A. L. De Lacey, M. Malki, *Int Microbiol*, **14**(2), 73 (2011)**.**
19. D. Majumder, J.P. Maity, Ch.-Y. Chen, Ch.-Ch. Chen, T.-Ch. Yang, Y.-F. Chang, D.-W. Hsu, H.-R. Chen, *International journal of hydrogen energy*, **39**, 21215 (2014).
20. J.M. Pisciotta, Z. Zaybak, D.F. Call, J.-Y. Nam, B.E. Logan, *Appl. Environ. Microb.* **78**(15). 5212 (2012).
21. N.J. Sacco, E.L.M. Figuerola, G. Pataccini, M.C. Bonetto, L. Erijman, E. Cortón, *Bioresource Technology*, **126**, 328 (2012).
22. B. Erable, N. Byrne, L. Etcheverry, W. Achouak, A. Bergel, *International journal of hydrogen energy*, **42**, 6059 (2017).
23. S.P. Jung, M.-H. Yoon, S.-M. Lee, S.E. Oh, H. Kang, J.-K. Yang, *Int. J. Electrochem. Sci.,* **9**, 315 (2014).
24. D.Z. Khater, K.M. El-Khatib, H. M. Hassan, *J. Gen. Engn Biotechn*., **15**, 127 (2017).
25. Q. Zhao, M. Ji, R. Li, Zh. J. Ren, *Bioresource Technology*, **237**, 178 (2017).
26. Y. Ueno, Y. Kitajima, *Advances in Microbiology*, **4**, 252 (2014).