ELECTRICITY GENERATION BY DIFFERENT MICROBIAL STRAINS

Marina Nicolova, Irena Spasova, Plamen Georgiev, Stoyan Groudev

University of Mining and Geology "St. Ivan Rilski", 1700 Sofia; mnikolova@mgu.bg

ABSTRACT. Microbial strains of iron reducing bacteria from the genera *Geobacter* and *Shewanella* were used in experiments for electricity generation by means of a microbial fuel cell. The cell was a plexiglass cylindrical column 45 cm high and with 10 cm internal diameter. The cell consisted of two sections separated by a permeable barrier of glass wool of 5 cm thickness. The feed stream, i.e. the solution subjected to treatment by the microorganisms, was supplied to the bottom anodic section of the column and the effluents passed through the cathodic section and exited at the top. Air was injected to the cathodic section. Several microbial strains from the two genera mentioned above were tested in these experiments. Some of the microbial strains, even those from the same taxonomic species, differed considerably-in their ability for electricity generation.

Keywords: electricity generation, microorganisms, iron reducing bacteria

ГЕНЕРИРАНЕ НА ЕЛЕКТРИЧЕСТВО ЧРЕЗ РАЗЛИЧНИ МИКРОБНИ ЩАМОВЕ

Марина Николова, Ирена Спасова, Пламен Георгиев, Стоян Грудев
Минно геоложки университет „Св. Иван Рилски", София 1700; mnicolova@mgu.bg

РЕЗЮМЕ. Микробни щамове на желязо редуциращи бактерии от родовете *Geobacter* и *Shewanella* са използвани за производство на електричество с помощта на микробна горивна клетка. Клетката представляваше плексигласова цилиндрична колона с височина 45 см и вътрешен диаметър 10 см. Клетката се състоеше от две секции, разделени от пропусклива преграда от стъклена вата с дебелина 5 см. Захранващият поток, т.е. разтворът, подложен на обработка от микроорганизмите, се подаваше в долния аноден участък на колоната и изтичащите вещества преминават пред катодната секция и излизат в горната част. Въздух се инжектираше в катодната секция. В експериментите бяха тествани няколко микробни щама, като дори тези от един и същ таксономичен вид, значително се различаваха по способността си за производство на енергия.

Ключови думи: генериране на електричество, микроорганизми, желязо редуциращи бактерии

Introduction

The natural contacts of rainfalls with heaps, dumps and even with the relatively stable deposits of the relevant ores are connected with the presence, growth and activity of different microorganisms. Some of these microorganisms, mainly the chemolithotrophic bacteria and archaea, are the prevalent inhabitants of these biotopes. At present, such microorganisms are largely used for leaching of useful components from different mineral substrates such as low-grade ores and mineral wastes but also concentrates and rich ores. These technologies are connected with efficient measures for conservation of the nature and its inhabitants. A special attention is paid to the development of efficient technologies for the electricity generation by means of microorganisms.

The ability of some microorganisms to generate electricity in constructed fuel cells is based on the microbial transfer of electrons from different organic substrates to the surface of electrodes located in the anoxic sections of the relevant fuel cells. Some of these microorganisms (mainly bacteria and archaea) are able to form biofilms on the anodic surface and to transfer the electrons directly, through microscopic pipes located in the pili of the microbial surface. The most active microorganisms using this mechanism are some bacteria possessing the so called anaerobic iron respiration. These bacteria are typical heterotrophs able to remove electrons from some organic compounds and to transfer them via microbial respiratory chains to ferric ions acting as final electron acceptors. The most studied from these bacteria are some species related to the genera *Geobacter* and *Shewanella*. They are able to form stable biofilms on the anodic electrodes in the constructed microbial fuel cells. In these cells, the electrons are removed from the anode by means of wires (usually from copper) and are transferred to contact with a resistance located outside the anodic section. As a result of such treatment, a portion of the chemical energy of electrons is converted to electricity and the electrons reach the aerobic cathodic section of the fuel cell in which they react with the protons to form water molecules (Rabaey and Verdtraete, 2005; Lovely, 2008).

Apart from the iron-reducing bacteria mentioned above, anaerobic microorganisms using sulphates as electronic acceptors and the typical fermenting bacteria are also used in investigations of this type.

This article is related to investigations on the ability of typical iron reducing bacteria to generate electricity by means of a constructed fuel cell.

Materials and Methods

The microorganisms used in this investigation were isolated from a natural wetland located in a territory in the proximity of Sofia. The experiments for electricity generation presented in this study were performed in the laboratories of the University of Mining and Geology in Sofia.

The constructed wetland was a plastic basin 1.50 m long, 1.2 m wide and 0.6 m deep. The bottom of the wetland was covered by a 0.2 m thick layer, consisting of a mixture of soil rich in biodegradable organic compounds, plant compost, manure, crushed limestone and sand. A permeable barrier consisting of the mixture mentioned above and with the following dimensions - 0.6 m height, 1.2 m width and 0.5 – 0.2 m length (at the bottom and the top, respectively), was constructed in the wetland perpendicularly to the direction of water flow.

The wetland was characterised by abundant vegetation and diverse microflora. *Typha angustifolia*, *Phragmites australis* and *Scirpus lacustris* were the dominant species in the wetland but representatives of the genera *Juncus*, *Eleocharis*, *Potamogeton* and several algae (mainly from the genera *Scenedesmus* and *Eudoria*) were also present.

Microbial strains of iron reducing bacteria from the genera *Geobacter* and *Shewanella* were used in experiments for electricity generation by means of microbial fuel cell. The cell was a plexyglass cilyndrical column 45 cm high and with internal diameter of 10 cm. The cell consisted of two sections separated by means of a permeable barrier of glass wool of 5 cm thickness. The feed stream, i.e. the solution subjected to treatment by microorganisms, was supplied to the bottom anodic section of the column and the effluents passed through the cathodic section located in the top part of the column and exited outside. Air was injected to the cathodic section. Several microbial strains from the two genera mentioned above were tested in these experiments. Some of these strains, even such from the same taxonomic species, differed considerably in their ability for electricity generation.

The elemental analysis of the waters was performed by means of atomic absorption spectrometry and inductively coupled plasma spectrometry.

The isolation, identification and enumeration of the microorganisms was carried out by the classical physiological and biochemical tests (Karavaiko et al., 1988) and by the molecular PCR methods (Sanz and Köchling, 2007; Escobar et al., 2008; Dopson and Johnson, 2012). The measurement of the produced power was carried out by digital multimeter.

Results and Discussion

The treatment of the polluted waters by means of the constructed wetland was connected with an efficient cleaning of these waters (Table 1). The content of the toxic elements (mainly heavy metals and arsenic) was decreased below the permissible levels (Ordinance № 12, 2002). At the same time the content of dissolved organic was considerably increased even above the relevant permissible level. The pH was also increased but within the relevant levels. These changes were related to the deep changes of the microorganisms present in these waters (Table 2). The numbers of acidophilic chemolithotrophs (mainly *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*) was negligible and in some samples these acidophiles were not even present.

Table 1. *Data about the polluted waters before and after the treatment by means of constructed wetland*

Parameters	Before treatment	After treatment	Permissible levels
pH	1.90 – 2.84	6.20 – 7.38	6 – 9
Solids, mg/l	48 – 176	28 – 60	100
Diss. O_2, mg/l	1.4 – 2.1	0.2 – 0.5	20
Diss. org. C, mg/l	2.3 – 8.2	45 – 82	40
Sulphates, mg/l	415 – 1085	215 – 392	400
Fe, mg/l	314 – 1085	1.4 – 6.8	5
Cu, mg/l	4.1 – 23	0.21 – 0.42	0.5
Mn, mg/l	14 – 41	0.2 – 0.8	0.8
Zn, mg/l	11 – 51	0.28 – 0.59	10
Cd, mg/l	0.2 – 0.8	< 0.01	0.02
As, mg/l	0.8 – 6.4	< 0.1	0.2

Table 2. *Microflora of the polluted waters before and after their treatment by means of the constructed wetland*

Microorganisms	Before treatment	After treatment
	Cells/ml	
Fe^{2+} - oxidising chemolithotrophs	$10^5 – 10^8$	$0 – 10^2$
S^0 - oxidising chemolithotrophs	$10^5 – 10^7$	$0 – 10^3$
Aerobic heterotrophic bactria	$10^1 – 10^3$	$10^2 – 10^4$
Cellulose-degrading aerobes	$0 – 10^2$	$10^1 – 10^4$
Anaerobic heterotrophic bacteria	$0 – 10^3$	$10^2 – 10^3$
Cellulose-degrading anaerobes	$0 – 10^1$	$10^4 – 10^6$
Sulphate-reducing bacteria	$0 – 10^2$	$10^6 – 10^8$
Fe^{3+} - reducing bacteria	$0 – 10^2$	$10^3 – 10^6$

At the same time the numbers of some microorganisms were considerably increased. The sulphate-reducing bacteria and the ferric iron reducing bacteria were the dominant microorganisms in the waters subjected to treatment by means of the constructed wetland. These microorganisms were very efficient for electricity generation in the microbial fuel cells (Table 3).

Table 3. *Maximum Fe^{3+} - reducing rates by means of different mesophilic iron-reducing bacteria*

Iron-reducing bacteria	Number of the strains tested	Maximum Fe^{3+} - reducing rate, mg/l.24 h
Shewanella genus		
S. odeinensis	4	123 – 335
S. loihica	10	140 – 321
S. putrefaciens	5	87 – 235
S. alga	12	77 – 182
Geobacter genus		
G. ferrireducens	10	125 – 203
G. metallireducens	6	140 – 212
G. sulfurreducens	6	62 – 140
G. hydrogenofilus	4	41 - 104

It must be noted that the highest production of electricity by such cells was achieved by means of mixed populations of sulphate-reducing and ferric iron reducing bacteria (Table 4).

Table 4. *Electricity generation by means of different anaerobic microorganisms present in the anodic section of the microbial fuel cell*

Microbial populations	Cells/ml	COD, mg O_2/l.h	Power, mW/m^2
Sulphate-reducing bacteria	>5.10^8	680 – 2350	2150 – 3750
Ferric iron-reducing bacteria	>3.10^8	750 – 1940	1540 – 2860

Mixed sulphate-reducing bacteria and iron-reducing bacteria	$>3.10^8$	820 – 2640	1580 – 4060
Mixed populations of anaerobic bacteria	$>5.10^8$	440 – 1720	1420 – 3140

Conclusion

The investigations on the electricity generation by the constructed microbial fuel cell revealed that this process was possible by using different anoxic microorganisms (sulphate-reducing, ferric iron-reducing and mixed populations of these anaerobic bacteria). It must be noted that some strains of these bacteria differ considerably from other strains of the same species. These conclusions reveal that the selection of the more active strains from the different microbial taxonomic species is an efficient way to make this approach very efficient for the future industrial application.

Acknowledgements. The authors would like to express their gratitude to the Scientific Research & International Partnership Unit of the University of Mining and Geology "St. Ivan Rilski", Sofia (under the frame of GPF-229/2020 project) for the financial support for publishing of this article.

References

Dopson, M. and Johnson, D., 2012. Biodiversity, metabolism and applications of acidophilic sulphur-metabolizing microorganisms, *Environ. Microbiol.*, 14, 2620 – 2631.

Escobar, B., Bustos K., Morales, G. and Salazar, O., 2008. Rapid and specific detection of *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* by PCR, *Hydrometallurgy*, 92, 102 – 106.

Karavaiko, G.I., Rossi, G., Agate, A.D., Groudev, S.N. and Avakyan, Z.A. (eds.), Biogeotechnology of Metals, *Manual GKNT Centre for International Projects*, Moscow, 1988.

Lovely, D.R., 2008. The microbe electric conversation of organic matter to electricity, *Current Opinion in Biotechnology*, 19, 1 – 8.

Ordinance № 12, 2002. Quality requirements for surface waters intended for drinking and domestic water supply, *State Gazette* 63, 2002, Corr. SG 15, 2012.

Rabaey K. and Verdtraete W., 2005. Microbial fuel cells: novel biotechnology for energy generation, *Trends in Biotechnology,* 23, 291 – 299.

Sanz, J.L. and Köchling, T., 2007. Molecular biology techniques used in wastewater treatment: An overview. *Process Biochemistry* 42, 119–133.