# ELECTRICITY GENERATION IN MICROBIAL FUEL CELLS BY MEANS OF DIFFERENT MICROORGANISMS

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**ABSTRACT.** Waters polluted with iron and some non-ferrous metals (Cu, Zn, Co) were subjected to treatment by means of permeable reactive multibarrier. The effluents from the multibarrier contained the metals in much lower concentrations but were enriched in dissolved organic compounds and contained anaerobic microorganisms from different physiological groups: mixed populations of mesophilic sulphate-reducing bacteria, mainly from the genera *Desulfovibrio, Desulfobacter,* and *Desulfomonas*; mixed populations of mesophilic iron-reducing bacteria of the genera *Geobacter* and *Shewanella*; mixed populations of mesophilic fermenting bacteria, mainly from the genera *Bacillus, Pseudomonas* and *Clostridium*. The electricity generated by these microorganisms in a two-section fuel cell varied from 14 to 510 $mW/m^2$.

**Keywords:** acid drainage, microorganisms, electricity generation

**ГЕНЕРИРАНЕ НА ЕЛЕКТРИЧЕСТВО В МИКРОБНА ГОРИВНА КЛЕТКА ЧРЕЗ РАЗЛИЧНИ МИКРООРГАНИЗМИ**

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**РЕЗЮМЕ.** Води, замърсени с желязо и някои цветни метали (Cu, Zn, Co) бяха подложени на обработка посредством пропусклива реактивна мултибариера. Разтворите, изтичащи от мултибариерата съдържаха метали в много по-ниски концентрации, но бяха обогатени на разтворени органични съединения и съдържаха анаеробни микроорганизми от различни физиологични групи: смесени популации на мезофилни сулфат-редуциращи бактерии, основно от родовете *Desulfovibrio, Desulfobacter* и *Desulfomonas*; смесени популации на мезофилни редуциращи желязо бактерии от родовете *Geobacter* и *Shewanella*; смесени популации на мезофилни ферментиращи бактерии, главно от родовете *Bacillus*, *Pseudomonas* и *Clostridium*. Електроенергията, генерирана от тези микроорганизми в дву-секционната горивна клетка, варираше от 14 до 510 $mW/m^2$.

**Ключови думи**: кисели дренажни води, микроорганизми, генериране на електричество

## Introduction

The treatment of polluted waters, including such containing very toxic elements (heavy metals, radionuclides, arsenic), by means of different passive systems has considerably increased in the past years due to its effectiveness and the acceptable economic evaluation. In most cases, such treatment is performed by means of permeable reactive multibarriers of different types. The effluents from the multibarriers usually contain the heavy metals and other toxic elements in much lower concentrations than the waters subjected to cleaning but are usually enriched in dissolved biodegradable organic compounds. Furthermore, the microflora of the treated waters usually contains various anaerobic microorganisms most of which participate in the process of water cleaning. Such waters and their microbial inhabitants are very suitable for electricity generation by means of microbial fuel cells (Barton and Fauque, 2007; Carver et al., 2011; Dopson and Johnson, 2012; Loveley, 2008).

The present paper contains data about the treatment of waters polluted with iron and some non-ferrous metals by means of permeable reactive multibarriers inhabited by different anaerobic microorganisms from different physiological groups: mixed populations of mesophilic sulphate-reducing bacteria, mixed populations of mesophilic iron-reducing bacteria, and mixed populations of mesophilic fermenting bacteria (all of them were most active at about 37°C); a mixed population of moderate thermophilic sulphate-reducing bacteria from the genus *Thermosulpobacterium* with an optimum temperature of about 55°C, and mixed populations of extreme thermophilic archaea from the genus *Archaeoglobus* with an optimum temperature of about 86°C. All these microorganisms were used in experiments for electricity generation by means of microbial fuel cells at the optimum temperatures relevant for each of them.

## Materials and Methods

Acid drainage waters polluted with iron and some non-ferrous metals (mainly Cu, Zn, Cd) were subjected to treatment by means of permeable reactive multibarriers. The multibarriers were plastic cylindrical columns 120 cm high, with an internal diameter of 30 cm. Three columns of this type were filled with a mixture of limestone (crushed to particle size of minus 10 mm) and biodegradable organic matter consisting of spent mushroom compost, fresh leaf compost, animal manure and saw dust. The columns were inoculated by water and soil samples inhabited by their relevant viable microflora. This microflora contained different anaerobic microorganisms: mixed populations of mesophilic sulphate-reducing bacteria, mixed populations of mesophilic iron-reducing bacteria, mixed populations of mesophilic bacteria fermenting different organic substrates, a mixed population of moderate thermophilic sulphate-reducing bacteria, and mixed populations of extreme thermophilic archaea.

Apart from the experiments on the isolation of natural representatives of microorganisms suitable for testing their ability to generate electricity, some already selected electrochemically active microorganisms were also used in this investigation. The microorganisms were added to a nutrient solution containing some biologically essential elements mentioned in the well-known 9 K nutrient medium (Silverman and Lundgren, 1959) but also containing various combinations of soluble biodegradable organic compounds, vitamins, and trace elements. Such solutions were subjected to continuous-flow circulation from the inlet to the outlet of the microbial fuel cells used for electricity generation.

The microbial fuel cells used in these investigations were of the type already efficiently used in other projects connected with the microbial generation of electricity. The cells were Plexiglas cylindrical columns 80 cm high, with an internal diameter of 12 cm. A perforated slab graphite-$Mn^{4+}$ anode and a graphite-$Fe^{3+}$ cathode were located in the bottom and in the top sections of the column, respectively. The two sections were separated by a permeable barrier of 5 cm thickness consisting of a 2.5 cm layer of glass wool and a 2.5 cm layer of glass beads. The feed stream containing the potential energy sources for the microorganisms was supplied to the bottom anodic section of the column and the effluents passed through the cathode section and continuously exited at the top. Biodegradable organic substrates of different types (proteins, lipids, sugars added separately or in different combinations) were used in these studies. Air was injected during the treatment to the cathode section.

Elemental analysis was done by atomic adsorption spectrometry and inductively coupled plasma spectrometry. The isolation, identification and enumeration of microorganisms were carried out by means of the classical physiological and biochemical tests (Karavaiko et al., 1988) and by the molecular PCR methods (Sanz and Köchling, 2007; Escobar et al., 2008).

## Results and Discussion

The treatment of the polluted acid drainage by means of the permeable reactive multibarrier was very efficient for removing the heavy metals and, at the same time, was connected with a considerable increase in the content of biodegradable organic compounds in the waters treated in this manner (Table 1). This increase was due to the biodegradation of the initial organic matter present in the multibarriers by means of the different heterotrophic microorganisms inhabiting the multibarriers and acting at different temperatures. The microflora in the multibarrier maintained at 37°C consisted mainly of mesophilic sulphate-reducing and iron-reducing bacteria but several fermenting heterotrophic bacteria (mainly of the genera *Bacillus*, *Pseudomonas* and *Clostridium*) were also present in relatively high concentrations (within $10^3$ – $10^5$ cells/ml).

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

| Parameters | Before treatment | After treatment | | |
|---|---|---|---|---|
| | | at 37°C | at 55°C | at 86 °C |
| pH | 1.45 - 3.50 | 6.80 - 7.25 | 6.95 - 7.40 | 6.90 - 7.53 |
| Eh, mV | (+370)-(+550) | (-170)-(-280) | (-175)-(-260) | (-170)-(-250) |
| TDS, mg/l | 3520 - 5720 | 590 - 1380 | 530 - 1250 | 510 - 1340 |
| Diss. $O_2$, mg/l | 1.0 – 2.1 | 0.1 – 0.2 | 0.1 | 0.1 – 0.3 |
| Diss. org. C, mg/l | 1.7 – 3.5 | 240 - 460 | 230 - 450 | 250 - 410 |
| Sulphate, mg/l | 590 – 1850 | 190 - 320 | 250 - 440 | 260 - 480 |
| Cu, mg/l | 8.2 – 28 | <0.1 – 0.5 | <0.1 - 0.2 | <0.1 - 0.3 |
| Zn, mg/l | 14 – 32 | <0.1 – 0.4 | <0.1 - 0.3 | <0.1 - 0.3 |
| Co, mg/l | 2.8 – 12 | <0.1 – 0.3 | <0.1 - 0.3 | <0.1 - 0.3 |
| Cd, mg/l | 0.1 – 0.5 | <0.02 | <0.01 - 0.03 | <0.01 - 0.03 |
| Mn, mg/l | 10 – 23 | 0.3 – 0.5 | 0.2 - 0.3 | 0.2 - 0.3 |
| Fe, mg/l | 550 - 1670 | 5.1 - 8.2 | <5 | <5 |

With respect to the electricity generation among the various mesophilic bacteria tested in this study, the most active were some mesophilic sulphate-reducing bacteria (Table 2). It must be noted, however, that the different strains related to one and the same taxonomic species can differ considerably from each other with respect to their ability to generate electricity (in fuel cells of this type, at least). The efficiency of the mixed cultures of sulphate-reducing bacteria consisting from representatives of different species of such bacteria was also variable within a large range. Some strains of the *Desulfovibrio desulfuricans* and *Desulfobulbus elongatus* were the most active with respect to electricity generation not only among the sulphate-reducing bacteria but also among all microorganisms tested in this study.

Table 2.
*Electricity generation by means of mesophilic sulphate-reducing bacteria*

| Sulphate-reducing bacteria | Power, mW/m$^2$ |
|---|---|
| *Desulfovibrio desulfuricans* | 203 – 510 |
| *Desulfobacter multivorans* | 125 – 404 |
| *Desulfobulbus elongatus* | 174 – 425 |
| *Desulfotomaculum nigrificans* | 65 – 212 |
| *Desulfococcus postgatei* | 25 – 170 |
| *Desulfosarcina variabilis* | 14 – 95 |
| Mixed cultures | 105 – 475 |

*Note: Treatnment at 37°C.*

Some of the iron-reducing bacteria were also very active with respect to their ability to generate electricity (Table 3). The different taxonomic species related to the genera *Shewanella* and *Geobacter* included strains quite different with respect to this ability. It must be noted, however, that some of the mixed cultures consisting of different species of iron-reducing bacteria were the most active among all strains and species from the two genera used in this study.

Table 3.
*Electricity generation by means of mesophilic iron-reducing bacteria*

| Iron-reducing bacteria | Power, mW/m$^2$ |
|---|---|
| *Shewanella loihica* | 170 – 321 |
| *S. odeinensis* | 154 – 335 |
| *S. putrefaciens* | 20 – 215 |
| *S. alga* | 95 – 190 |
| *Geobacter metallireducens* | 161 – 212 |
| *G. ferrireducens* | 140 – 192 |
| *G. sulfurreducens* | 91 – 132 |
| *G. hydrogenofilus* | 48 – 97 |
| Mixed cultures | 125 – 325 |

*Note: Treatnment at 37$^o$C.*

As a whole, the electricity generation by means of different species, strains and mixed cultures of moderate thermophilic sulphate-reducing bacteria at 55$^o$C (Table 4) was lower in comparison with the electricity generation by means of mesophilic bacteria at 37$^o$C. However, it must be noted that the numbers of microbial species and strains of the mesophilic bacteria used in this study were much higher than the relevant numbers connected with the moderate thermophilic sulphate-reducing bacteria used.

Table 4.
*Electricity generation by means of moderate sulphate-reducing bacteria*

| Sulphate-reducing bacteria | Power, mW/m$^2$ |
|---|---|
| *Thermodesulfobacterium sp.* | 55 – 170 |
| *T. commune* | 68 – 194 |
| *T. mobilis* | 51 – 212 |
| Mixed cultures | 86 – 280 |

*Note: Treatnment at 55$^o$C.*

The electricity generation by means of sulphate-reducing archaea at 86$^o$C was also lower (Table 5) than that achieved at 37$^o$C by means of the mesophilic sulphate-reducing bacteria. A serious reason for this is probably the same one mentioned above: about the comparative data obtained by means of the mesophilic and moderate thermophilic bacteria (i.e. the much lower number of investigations on the electricity generation by means of thermophilic microorganisms performed until now in comparison with that performed by means of mesophiles).

Table 5.
*Electricity generation by means of moderate sulphate-reducing bacteria*

| Sulphate-reducing bacteria | Power, mW/m$^2$ |
|---|---|
| *Thermodesulfobacterium sp.* | 120 – 235 |
| *T. commune* | 73 – 203 |
| *T. mobilis* | 60 – 187 |
| Mixed cultures | 51 – 210 |

*Note: Treatnment at 86$^o$C.*

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