BIOREMEDIATION OF ACID DRAINAGE WATERS FOLLOWED BY ELECTRICITY GENERATION

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ABSTRACT. Acid drainage waters generated during the leaching of a copper-bearing sulphide ore and containing toxic heavy metals and arsenic were subjected to treatment by means of a permeable reactive multibarrier under laboratory conditions. The multibarrier was filled by a mixture of crushed limestone and biodegradable organic matter (spent mushroom compost, fresh leaf compost, animal manure and sawdust) and was inhabited by a viable indigenous microflora consisting of various anaerobic microorganisms, mainly sulphate- and iron-reducing bacteria and other interconnected microorganisms. An efficient cleaning of the polluted waters was performed in the multibarrier, mainly by the precipitation of the heavy metals and arsenic as the respective insoluble sulphides. Apart from the natural microflora of the multibarrier effluents, an inoculum consisting of electrochemically selected active microorganisms was added to these effluents. Portions of those were treated in a microbial fuel cell under continuous-flow conditions and this resulted in the generation of electricity with a power of about 1800 – 2100 mW/m^2.

Keywords: acid drainage, microorganisms, electricity generation

БИОРЕМЕДИАЦИЯ НА КИСЕЛИ ДРЕНАЖНИ ВОДИ, ПОСЛЕДВАНА ОТ ГЕНЕРИРАНЕ НА ЕЛЕКТРИЧЕСТВО

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РЕЗЮМЕ. Кисели дренажни води, генерирани при излугване на медна сулфидна руда и съдържащи токсични тежки метали и арсен, бяха подложени на третиране посредством пропусклива реактивна мултибариера при лабораторни условия. Мултибариерата беше запълнена със смес от натрошен варовик и биологично разградима органична материя (отработен гъбен компост, свеж листвен компост, животински тор и стърготини) и беше обитавана от жизнена местна микрофлора, състояща се от различни анаеробни микроорганизми, главно сулфатредуциращи и желязоредуциращи бактерии и други взаимно свързани микроорганизми. Ефикасно пречистване на замърсените води беше извършено в мултибариерата, главно чрез утаяване на тежките метали и арсена като съответните неразтворими сулфиди. Освен естествената микрофлора на изтичащите от мултибариерата води, една закваска, съдържаща селекционирани електрохимично активни микроорганизми, беше добавена към тези води. Части от тях бяха третирани в микробна горивна клетка при условия на непрекъснат поток и това доведе до генериране на електричество с мощност около 1800 – 2100 mW/m^2.

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

Introduction

The acid drainage waters generated under natural conditions and, very often, as a result of the human activity connected with the recovery and processing of sulphide minerals (mainly pyrite) constitute a very serious environmental problem. This is due to the fact that in most cases, apart from the sulphuric acid, such waters contain different toxic elements, like heavy metals, radionuclides and arsenic. The prevention of this process is usually much more desirable than the subsequent treatment of such waters. Unfortunately, the efficient prevention of acid generation in the huge dumps of low-grade waste ores that are rich-in-sulphides, or of other mineral wastes is usually an extremely difficult and costly process. In some cases, even after total remediation of the environment, it is necessary to treat the polluted drainage waters arising mainly as a result of the activity of some of the indigenous microorganisms inhabiting the relevant territory. Such treatment can be connected with the extraction of some valuable components, like some non-ferrous metals and rare earth elements, and even with the production of electricity by means of especially constructed microbial fuel cells (Spasova et al., 2014, 2016).

This paper contains some data about the possibility to combine the treatment of acid drainage waters generated in mining waters rich in pyrite with the production of electricity by means of a laboratory-scale fuel cell.

Materials and Methods

Acid drainage waters generated during the bioleaching of copper-bearing sulphide ore and containing toxic heavy metals and arsenic were subjected to treatment by means of a permeable reactive multibarrier under laboratory conditions. The multibarrier was a plastic cylindrical column, 120 cm high, with an internal diameter of 30 cm. The column was 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 column was inhabited by a viable microflora derived from the natural inhabitants of these organic substrates. This microflora consisted of different anaerobic microorganisms, mainly sulphate-reducing bacteria and other interconnected microbial species. Apart from the natural microflora of the organic substrates mentioned above, an inoculum containing electrochemically selected active

microorganisms was also added to the multibarrier effluents, together with a nutrient solution containing biologically essential elements (a combination of soluble biodegradable organic compounds, vitamins, and trace elements). Such solutions were subjected to continuous-flow circulation from the inlet to the outlet of a microbial fuel cell.

The microbial fuel cell was a Plexiglas cylindrical column, 80 cm high, with a 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 2.5 cm layer of a glass beads. The feed stream, i.e. the effluents from the multibarrier, was supplied to the bottom anodic section of the column and the effluents passed through the cathodic section and continuously exited at the top. Air was injected during the treatment to the cathodic section.

In the course of time, a biofilm consisting of a consortium of different electrochemically active microorganisms was formed on the anode electrode.

The quality of the waters being treated by means of the permeable reactive multibarrier and by the microbial fuel cell was monitored at different sampling points. Those points were located at the inlet and the outlet of these components of the system for the water cleaning and electricity generation. The parameters measured in situ included: pH, Eh, dissolved oxygen, total dissolved solids, and temperature. Elemental analysis was done by atomic adsorption spectrometry and by inductively coupled plasma spectrometry. The isolation, identification, and enumeration of microorganisms were 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).

Results and Discussion

The treatment of the polluted acid drainage waters by means of a permeable reactive multibarrier was very efficient and, in most cases, the acidity and the contents of toxic heavy metals, arsenic and sulphates were decreased below the relevant permissible levels (Tables 1 and 2).

Table 1.
Data about the acid drainage waters before and after their treatment by the permeable multibarrier

Parameters	Before treatment	After treatment	Permissible levels
pH	1.70 - 2.51	6.82 - 7.10	6 - 9
Eh, mV	(+325)-(+512)	(-170)-(-264)	–
TDS, mg/l	2,420 – 5,540	512 – 1,250	1,500
Diss. O_2, mg/l	1.2 – 2.3	0.1 – 0.3	2
Diss. org. C, mg/l	1.7 – 3.2	247 – 486	20
Sulphate, mg/l	640 - 1,544	240 – 415	400
Cu, mg/l	3.7 – 17	<0.05 – 0.32	0.5
Zn, mg/l	9.9 – 37	<0.05 – 0.46	10
Cd, mg/l	0.05 – 0.21	<0.01	0.02
Mn, mg/l	7.1 – 5.3	0.14 – 0.08	0.8
Fe, mg/l	545 – 1,270	2.3 – 6.2	5
As, mg/l	0.05 - 0.28	<0.01	0.2

Table 2.
Microflora of the acid drainage waters before and after their treatment by means of the permeable multibarrier

Microorganisms	Before treatment	After treatment
	Cells/ml	
Acidithiobacillus ferrooxidans	10^7 - 10^9	–
Acidithiobacillus thiooxidans	10^4 – 10^7	–
Leptospirillum ferrooxidans	10^5 – 10^7	–
Aerobic heterotrophic bacteria	10^2 – 10^5	–
Fungi	0 – 10^3	–
Anaerobic heterotrophic bacteria	0 – 10^3	10^6 – 10^8
Denitrifying bacteria	0 – 10^1	10^4 – 10^6
Sulphate-reducing bacteria	0 – 10^3	10^6 – 10^8
Fe^{3+} - reducing bacteria	0 – 10^2	10^2 – 10^4
Mn^{4+} - reducing bacteria	0 – 10^2	10^2 – 10^4
As^{5+} - reducing bacteria	0 – 10^1	10^1 – 10^2
Methanogenic bacteria	0 – 10^1	10^2 – 10^3

At the same time, the content of biodegradable organic carbon in the multibarrier effluents was increased considerably. This made the treatment of these effluents in the microbial fuel cell very efficient (Table 3).

Table 3.
Treatment of the multibarrier effluents by means of the microbial fuel cell

Parameters	Values
Influents in the MFC:	
COD, mgO_2/l.h	520 – 1,650
SO_4^{2-}, mg/l	240 – 1,040
COD/SO_4^{2-}ratio	1.6 – 4.4
pH	7.10 – 7.45
Eh, mV	(-210) - (-255)
Temperature, ^oC	28 – 35
Heavy metals concentrations	Below permissible levels[x]
Voltage of the open circuit, mV	140 – 280
O_2 dissolved in the cathodic section, mg/l	7.7 – 8.2
Power, mW/m^2	1,800 - 2,100

Note: [x] – With the exception of iron – up to 50 mg/l

The microflora in the anodic section of the microbial fuel cell (Table 4) contained considerable amounts of microorganisms able to transfer electrons from the dissolved organic substrates to the anode electrode located in the anoxic section of the microbial fuel cell. The main role in the process was played mainly by two different groups of anaerobic microorganisms: the sulphate-reducing and the iron-reducing bacteria.

Table 4.
Microorganisms in the anaerobic anodic section of the microbial fuel cell

Microorganisms	Cells/ml
Anaerobic heterotrophic bacteria (pH 7)	10^7 – 10^9
Fermenting bacteria	10^6 – 10^8
Sulphate-reducing bacteria	10^7 – 10^8
Denitrifying bacteria	10^4 – 10^5
Fe^{3+} - reducing bacteria	10^7 – 10^8
Mn^{4+} - reducing bacteria	10^4 – 10^6
As^{5+} - reducing bacteria	10^3 – 10^5
Methanogenic bacteria	0 – 10^2

The sulphate-reducing bacteria were able to perform the electron transport by means of secreted metabolites, mainly by the hydrogen sulphide. These microorganisms were related to different genera and species (Table 5) and differed from each other mainly in the rate of their anoxic respiration process, including in the rate of degradation of the different organic substates used as sources of electrons for the final acceptor, i.e. for the sulphate anion.

Table 5.
Sulphate-reducing bacteria in the anodic section of the microbial fuel cell

Sulphate-reducing bacteria	Cells/ml
Desulfovibrio (mainly *D. desulfuricans*)	$10^5 – 10^7$
Desulfobulbus (mainly *D. elongatus*)	$10^3 – 10^7$
Desulfococcus (mainly *D. postgatei*)	$10^2 – 10^5$
Desulfobacter (mainly *D. multivorans*)	$10^2 – 10^6$
Desulfotomaculum (mainly *D. nigrificans*)	$10^1 – 10^3$
Desulfosarcina (mainly *D. variabilis*)	$10^3 – 10^5$
Desulfomonas (non identified species)	$10^2 – 10^4$

It must be noted that some bacteria related to the genus *Pseudomonas*, which were also present in the mixed microbial consortium in the anoxic section of the microbial fuel cell, produced some dissolved organics which were used by the sulphate-reducing bacteria as donors of electrons in their own anoxic respiration process. The iron-reducing bacteria present in some of the microbial consortia in the anoxic section of the microbial fuel cell were related mainly to two different taxonomic genera: *Schewanella* and *Geobacter*. These bacteria were able to transfer electrons from the dissolved organic substrates in the anoxic section via their own respiratory chains and extracellular matrix directly to the anode located also in this section of the microbial fuel cell. It is known that some members of these two genera differ considerably from each other with respect to their mechanisms of the extracellular electron transport from the relevant organic substrates to the cytochromes of c-type present in their respiratory chains. It must be noted that even strains related to one and the same taxonomic species, the well-known *Schewanella oneidensis*, exhibit considerable differences in their current-generating mechanisms.

The iron(III)-reducing bacteria were also well present in the anodic section of the microbial fuel cell, although in relatively low concentrations than the sulphate-reducing bacteria (Table 6).

Table 6.
Ferric iron-reducing bacteria in the anodic section of the microbial fuel cell

Iron(III) – reducing bacteria	Cells/ml
Geobacter metallireducens	$10^4 – 10^7$
Geobacter sulfurreducens	$10^3 – 10^5$
Geobacter sp.	$10^2 – 10^4$
Schewanella oneidensis	$10^4 – 10^6$
Schewanella sp.	$10^2 – 10^4$

It must be noted that the reduction and growth of the iron(III)-reducing bacteria depend considerably not only on the type, composition, and concentration of the organic substrates but also on the structure and surface of the iron(II) minerals. The amorphous Fe(III)-oxyhydroxides are reduced at relatively high rates but the crystalline Fe(III)-oxide minerals, such as goethite [Fe(OH)] and hematite (Fe_2O_3), are reduced incompletely and at lower rates (Johnson and McGinness, 1991).

The results from this study demonstrate that the cleaning of acid drainage waters by means of a permeable multibarrier can be combined with the subsequent electricity generation by the treatment of multibarrier effluents that are rich-in-biodegradable organic compounds. Electricity generation is performed in a microbial fuel cell inhabited by electrochemically active microorganisms (mainly sulphate and iron reducing bacteria).

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